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# Identification of aroma active compounds in orange essence oil using gas chromatography–olfactometry and gas chromatography–mass spectrometry

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## Abstract

Using GC–MS and GC–flame ionization detection (FID)/olfactometry, 95 volatile components were detected in orange essence oil, of which 55 were aroma active. In terms of FID peak area the most abundant compounds were: limonene, 94.5%; myrcene, 1%; valencene, 0.8%; linalool, 0.7%, and octanal, decanal, and ethyl butyrate, 0.3% each. One hundred percent of the aroma activity was generated by slightly more than 4% of the total volatiles. The most intense aromas were produced by octanal, wine lactone, linalool, decanal,  $\beta$ -ionone, citronellal, and  $\beta$ -sinensal. Potent aroma components reported for the first time in orange essence oil include: *E*-2-octenal, 1-octen-3-ol, *Z*-4-decenal, *E,E*-2,4-nonadienal, guaiacol,  $\gamma$ -octalactone, and *m*-cresol. Over 20 compounds were identified for the first time in orange essence oil using MS, however, most did not exhibit aroma activity.

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**Keywords:** Oils; Olfactometry; Detection, GC; Food analysis; Aroma compounds; Volatile organic compounds

## 1. Introduction

Orange essence oil is a natural flavoring material obtained during the removal of water in orange juice in the process of making frozen orange juice concentrate. It is usually condensed in the first stage of an evaporator and separated from the aqueous portion by centrifugation. The composition and flavor quality of this product varies considerably depending on the orange cultivar, maturity, and processing conditions used to extract and concentrate the juice. Orange

essence oil is a complex mixture of aroma volatiles, but exactly which volatiles contribute to the overall aroma quality has not been established.

Gas chromatography–olfactometry (GC–O) is a hybrid method of determining aroma active compounds in foods, combining a high resolution chromatographic separation with human sensory detection of the GC effluent [1]. In time-intensity GC–O, a human assessor describes the odor quality, indicates the time and duration when an aroma is detected, and/or estimates aroma intensity. Whereas instrumental methods, such as GC–MS, can identify and quantify the most abundant volatiles in a sample, they provide no direct information as to which compounds have aroma activity. Aroma activity only

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occurs when a compound's concentration exceeds its aroma threshold in a given medium. Aroma thresholds for food volatiles can vary from hundreds of ppm to sub ppb [2]. Thus, compounds present in high concentration often provide little or no aroma activity, whereas some aroma volatiles found at trace concentrations may have intense aroma activity. If a compound can be quantified and its aroma threshold is known, aroma activity can be assumed if the ratio is greater than one. These ratios are often called aroma impact values and represent an indirect approach to determine aroma activity. Unfortunately, this approach does not work well in complex systems, as it is often difficult to quantify trace aroma active compounds because of incomplete resolution and instrument detection limits. Without a direct sensory technique to guide the investigator, it is impossible to determine which trace components possess aroma activity.

Since many food volatiles have little to no aroma activity, quantifying the compounds instrumentally could overemphasize unimportant compounds simply because they exist in high concentrations. Furthermore, critical aroma impact compounds are often overlooked because they exist at such low levels. For example, Le Guen et al. identified 85 volatiles in mussels, but only 33 had aroma activity. The compounds with highest aroma activity accounted for 12.5–18% of the volatiles identified in farmed and wild mussels, respectively [3]. Similarly, in *Citrus junos* peel oil, of 115 volatile compounds only 46 were aroma active. The 38 compounds with highest aroma activity accounted for less than 0.5% of the total volatiles identified in the sample [4].

As aroma intensity is a subjective measurement, based on human perception, methods to objectively quantify the aroma impact have been developed. The most common GC–O methods are time-intensity [5], dilution analysis [6,7], and detection frequency [8,9]. Time-intensity analysis employs 2–4 trained assessors who sniff the GC effluent, continuously indicating aroma intensity using a potentiostat whose output is recorded. A verbal descriptor is also recorded. This method has been employed to determine the aroma impact compounds in orange juice [10], wines [5], mussels [11], and wine must [12].

Flavor quality is important in the orange juice industry, where orange oils are used to partially

restore the flavors lost during thermal concentration, and in the flavor industry, where they are used in beverages, ice cream, chewing gum, and other candies. Flavoring materials are present in both orange juice and peel. Over 100 investigations concerning the volatile composition of orange juice as well as several recent GC–O studies of orange juice aroma [10,13–17] have been reported. Similarly, over 50 orange peel oil compositional studies as well as a few GC–O peel oil studies [4,18–20] have been reported. In contrast, few orange essence oil volatile studies [21–25] have been reported and no GC–olfactometry studies have been found.

Therefore, the primary objective of this study was to identify which compounds in commercial orange essence oil exhibit aroma activity and thus impact its overall sensory quality. GC–O retention index values from two dissimilar chromatographic columns along with aroma descriptors were used to tentatively characterize aroma active compounds and authentic standards were injected on both columns to positively identify them. Mass spectrometry (MS) was employed to confirm identification whenever possible. Since flavor is determined by the relative strength of aroma components, a secondary goal was to determine relative intensity between those compounds which demonstrate aroma activity. Relative volatile composition (as determined by flame ionization detection (FID) peak areas and identified by MS) was compared with relative aroma activity as determined by GC–O to identify which compounds do not directly contribute to orange essence oil aroma quality.

## 2. Materials and methods

### 2.1. Sample

Orange essence oil from Givaudan Flavors (Lakeland, FL, USA) was used. The oil was from the water-insoluble, lipophilic portion of the condensed distillate formed when orange juice is thermally concentrated. This commercial oil was a blended product and was stored at  $-10^{\circ}\text{C}$  until use. For analysis, the oil was diluted 1:1 in hexane.

## 2.2. Chemicals

Aroma standards were obtained from the following sources: ethyl 2-methylpropanoate, methional, 2-methyl-3-furanthiol, 4-hydroxy-2,5-dimethyl-3(2H)-furanone, vanillin,  $\gamma$ -decalactone, 1-octen-3-one, (*E*)-2-hexenal, (*E*)-2-nonenal, (*E*)-2-decenal, (*E*)-2-undecenal (*E,Z*)-2,6-nonadienal, (*E,E*)-2,4-nonadienal, (*E,E*)-2,4-decadienal were purchased from Aldrich (Milwaukee, WI, USA). 3-Mercaptohexyl acetate and 3-mercaptohexan-1-ol were bought from Interchim (Montlucon, France). Limonene, octanal, nonanal,  $\beta$ -caryophyllene, 1,8-cineole, citral, citronellal, citronellol, *p*-cymene, decanal, dodecanal, geraniol, nootkatone, octanal, octanol,  $\alpha/\beta$ -sinensal,  $\gamma$ -terpinene, valencene, (*Z*)-3-hexenal, nootkatone, 1,10-dihydro nootkatone, linalool, terpinen-4-ol were obtained as gifts from SunPure (Lakeland, FL, USA). *p*-1-Menthen-8-thiol and  $\gamma$ -damascenone were obtained from Givaudan. 4-Mercato-4-methyl-2-pentanone and 4-mercato-4-methyl-2-pentanol were synthesized in our laboratory. (*Z*)-2-Nonenal was found in purchased (*E*)-2-nonenal at 5–10% level and (*Z*)-2-decenal was found in purchased (*E*)-2-decenal at a similar level. (*E,Z*)-2,4-Nonadienal and (*E,Z*)-2,4-decadienal were, respectively, present in the purchased (*E,E*)-2,4-nonadienal and (*E,E*)-2,4-decadienal, while *trans*-4,5-epoxy-(*E*)-2-decenal was found in an oxidized sample of (*E,E*)-2,4-decadienal. Their identities were confirmed by mass spectra, retention indices and odor qualities. Wine lactone [3a,4,5,7a-tetrahydro-3,6-dimethyl-2(3H)-benzofuranone] was obtained as a gift from Professor Dr. G. Helmchen (University of Heidelberg, Heidelberg, Germany).

## 2.3. Gas chromatography

Gas chromatography was performed on a HP 5890 gas chromatograph (Hewlett-Packard, Palo Alto, CA, USA). A 0.2- $\mu$ l sample was injected splitless onto a capillary column (DB-wax, 30 m $\times$ 0.32 mm I.D., 0.5  $\mu$ m film thickness; J&W Scientific, Folsom, CA, USA). The temperature was programmed from 40 °C to 240 °C at 7 °C/min, with a 5-min final temperature hold. Injector temperature was 240 °C and FID temperature 250 °C with the effluent split 1:2 to the FID system and a sniffer port.

Samples were also analyzed using a DB-5 capillary column (30 m $\times$ 0.32 mm I.D., 0.5  $\mu$ m film thickness; J&W Scientific), programmed from 40 °C to 265 °C at 7 °C/min, 5 min final hold. Injector temperature was 220 °C and FID temperature 250 °C.

## 2.4. Olfactometry

Four olfactometry panelists, trained in GC-sniffing and odor recognition, sniffed the humidified effluent of the GC four times each. The intensity of each compound with aroma activity was recorded on a sliding scale from 0 to 15 and the aroma quality noted. The output of the variable potentiometer was connected to a separate channel in the ChromPerfect software used to gather GC-FID data, and time-intensity data were continuously recorded. The equipment and software used are detailed in Bazemore et al. [10]. A compound was deemed aroma active if it was detected in at least half of all sniffs (8 of 16 runs). The intensity of each run was normalized so the highest intensity had a score of 100. The normalized intensities of all the runs where the compound was detected were then averaged. If the compound was not detected in a run its value was treated as missing, not zero.

## 2.5. Mass spectrometry

A 0.2- $\mu$ l sample of undiluted orange essence oil was injected splitless into a Finnigan GCQ (San Jose, CA, USA) with a capillary column (DB-5, 60 m $\times$ 0.25 mm I.D., 0.25  $\mu$ m film thickness; J&W Scientific). Oven temperature was programmed from 40 °C to 250 °C at 7 °C/min with an initial hold time of 0.5 min and final hold time of 10 min. The MS (electron impact ionization) conditions were: ionization energy, 70 eV; mass range, 40–300 u; scan rate, 2 scans/s; electron multiplier voltage, 1050 V. Injector temperature was 200 °C and transfer line temperature 250 °C.

## 2.6. Compound identification

Aroma active compounds (Table 1) were identified by alkane linear retention indices ( $C_8$  to  $C_{25}$ ) [26] on DB-wax and DB-5 columns and odor quality. All aroma active compounds were confirmed

by injecting authentic standards on both columns and noting retention index and aroma. Library mass spectra [42] were also used where concentration allowed. Other volatile compounds (Table 2) were identified based on alkane linear retention indices ( $C_5$  to  $C_{25}$ ) on the DB-5 column and comparison of mass spectra with library spectra.

### 3. Results and discussion

#### 3.1. Identification and quantitation of aroma active compounds by GC–olfactometry

Ninety integratable FID peaks were observed in chromatograms of orange essence oil. The same sample produced 55 aroma peaks in the corresponding aromagram (Fig. 1). Their retention characteristics and descriptors are summarized in Table 1. Forty-three aroma compounds were identified. Nine were terpenes, 15 aldehydes, 10 alcohols, six ketones, and three esters.

Compounds which exhibited strongest aroma activity (in order of decreasing intensity) were: octanal, wine lactone, linalool,  $\beta$ -ionone, decanal,  $\beta$ -sinensal, and citronellal. These contributed citrusy, floral, sweet, and herbal notes to the oil. Nine terpenes were found to have aroma activity:  $\alpha$ -pinene,  $\beta$ -pinene, myrcene,  $\alpha$ -phellandrene, limonene,  $\beta$ -phellandrene,  $\gamma$ -terpinene, *p*-cymene, and  $\beta$ -caryophyllene, a sesquiterpene. As a group they impart a relatively low intensity musty, green, and minty aroma to the oil. Limonene is the single, overwhelmingly largest component of orange essence oil (94.5% of total peak area). A moderate aroma peak (see Table 1) was associated with it. However, earlier GC–O studies of orange juice [27] found trace aroma activity for this terpene. Another suggestion that this aroma peak associated with limonene is an impurity is the non-typical descriptor, licorice. Standard limonene is typically described as citrusy [13,15]. The licorice aroma was also observed in recent GC–O studies of processed orange peel oils [18]. The authors of this study concluded that the licorice aroma was not due to limonene, but a coeluting impurity.

Although present in relatively small amounts, oxygenated terpenes and products of unsaturated

fatty acid degradation produced the most aroma activity. Terpene aldehydes which have strong aroma activity are citronellal, neral, geranial, and  $\beta$ -sinensal (a sesquiterpene aldehyde). Many fatty acid degradation aldehydes: hexanal, *Z*-3-hexenal, octanal, nonanal, *Z*-4-nonenal, decanal, *Z*-4-decenal, *E*-2-decenal, *E,E*-2,4-nonadienal, dodecanal, and *E,E*-2,4-decadienal also exhibited intense aroma activity. The aldehydes contribute to the citrusy, floral, fatty and green aromas. The terpene alcohols: 1,8-cineole, linalool, terpinen-4-ol, citronellol, and geraniol, added fruity and minty aromas. The fatty acid degradation alcohols: hexanol, 1-octen-3-ol, and octanol, produced green, mushroom, and citrusy aromas. Fruity and floral smelling  $\beta$ -damascenone and  $\beta$ -ionone are carotenoid degradation products from the juice and mushroom smelling 1-octen-3-one is from fatty acid degradation. Sotolon (spicy) is formed from a reaction between ascorbic acid and ethanol [28].

While esters are the most important aroma compounds in several fruits [29–32], the oxygenated terpenes and medium length aldehydes are more important in citrus [33]. However, ethyl butyrate imparted a strong fruity note and wine lactone (a cyclic ester), was one of the key aroma impact compounds in orange essence oil and imparted a strong herbal, spicy note.

Methional, furaneol, 4-vinyl guaiacol, and vanillin are aroma active volatiles which have been reported in orange juice [10,13], but have not been found in cold pressed orange peel oil. These aroma active compounds are likely formed during heat treatment and/or storage of the juice and would thus not likely be found in cold pressed peel oil which does not undergo thermal processes. Since orange essence oil has gone through the same thermal process as the juice (in the production of concentrate), their presence might also be expected. However, these compounds have high boiling points (low volatility) and what little was volatilized and found in the condensate would probably partition into the water phase since they all have very high water solubilities. The fact that other high boiling compounds such as sotolon and guaiacol, are found in peel oil, could suggest that they were formed during the processing and storage of the oil.

Wine lactone, first reported in white wine [34] and

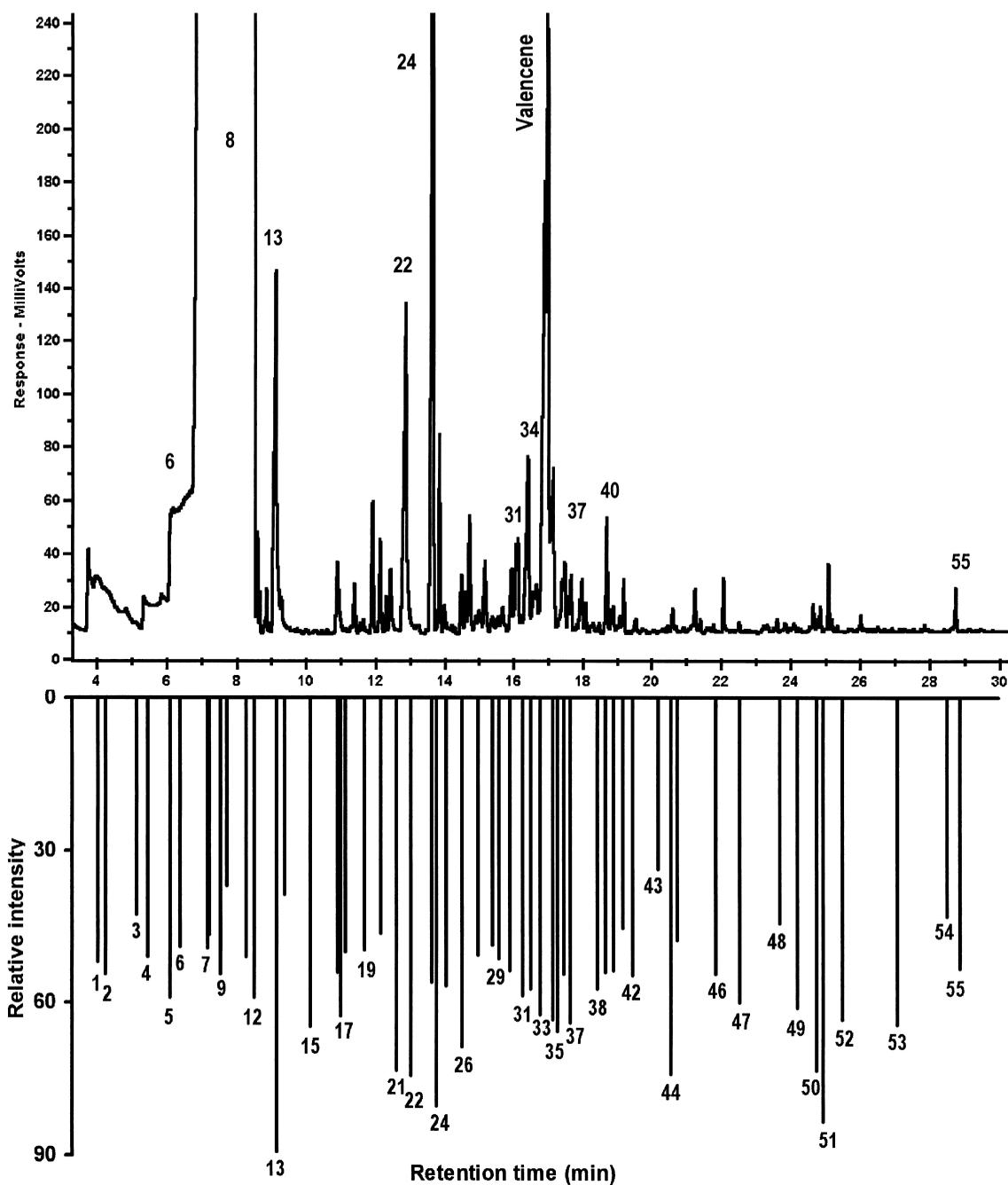


Fig. 1. GC-FID (top) and GC-olfactometry average of four runs by four panelists (bottom) of orange essence oil on DB-wax column. See Section 2 for chromatography details. Peak numbers refer to compounds in Table 1.

Table 1  
Aroma active compounds in orange essence oil determined by GC–O and GC–FID

No.	Compound	Linear retention index		Descriptors	Relative intensity <sup>a</sup>	FID area %	Previously reported <sup>b</sup>
		DBwax	DB-5				
1	$\alpha$ -Pinene <sup>c</sup>	1027	934	Fruity, piney	52	0.10	[13–15,17,19,20]
2	Ethyl butyrate <sup>c</sup>	1037	800	Fruity, sweet	55	0.30	[10,13–15,17,27]
3	Hexanal <sup>d</sup>	1093	784	Green	43	0.023	[13–15,17]
4	$\beta$ -Pinene <sup>c</sup>	1113	990	Musty, green	51	0.0063	[4,14,19,20]
5	Z-3-Hexenal <sup>c</sup>	1151	784	Grassy	59	0.045	[10,13,15]
6	Myrcene <sup>c</sup>	1176	994	Musty, geranium	49	1.04	[4,10,13–15,19,20]
7	$\alpha$ -Phellandrene <sup>d</sup>	1205	1032	Fruity, minty	49	<sup>c</sup>	[4,19]
8	Limonene <sup>c</sup>	1212	1036	Licorice	47	94.5	[4,10,13–15,17,19,20,27]
9	1,8-Cineole <sup>d</sup>	1224	1039	Minty	55	<sup>e</sup>	[10]
10	$\beta$ -Phellandrene <sup>d</sup>	1241	1053	Minty	37	<sup>e</sup>	
11	$\gamma$ -Terpinene <sup>c</sup>	1265	1074	Citrusy, terpeny	51	<sup>e</sup>	[4,17,19]
12	<i>p</i> -Cymene <sup>c</sup>	1277	1033	Lemon, fruity	59	<sup>e</sup>	[4,20]
13	Octanal <sup>c</sup>	1307	1004	Lemon, green	89	0.31	[4,10,13–15,17,18,20]
14	1-Octen-3-one <sup>d</sup>	1323	979	Mushroom	39	0.020	[13,15]
15	Hexanol <sup>d</sup>	1360	884	Fruity, green	65	0.0027	[17]
16	Nonanal <sup>c</sup>	1402	1103	Sweet, melon	54	0.064	[4,10,13–15,18,20]
17	Unknown	1405		Green, citrus	63	t <sup>f</sup>	
18	Unknown	1415		Mushroom	50	t	
19	<i>E</i> -2-Octenal <sup>d</sup>	1442	1056	Sweet, sour	50	0.0059	
20	1-Octen-3-ol <sup>d</sup>	1465	980	Mushroom	47	0.069	
21	Citronellal <sup>c</sup>	1488	1159	Lemon, citronella	73	0.044	[19,20]
22	Decanal <sup>c</sup>	1510	1211	Lemon, fatty	74	0.32	[10,13,15,17,18,20]
23	Z-4-Decenal <sup>d</sup>	1544	1200	Green, musty	56	t	
24	Linalool <sup>c</sup>	1551	1107	Floral	80	0.68	[4,10,13–15,17–20,27]
25	Octanol <sup>c</sup>	1565	1074	Herbal, fatty	57	0.084	[4,14,19]
26	Terpinen-4-ol <sup>c</sup>	1593	1179	Woody	69	0.037	[10,14]
27	$\beta$ -Caryophyllene <sup>c</sup>	1618	1467	Musty, green	51	0.027	[19]
28	Unknown	1641		Sweet, green	49	t	
29	<i>E</i> -2-Decenal <sup>c</sup>	1651	1261	Green	51	0.012	[20]
30	Unknown	1672		Minty	54	0.050	
31	Neral <sup>c</sup>	1690	1294	Citrus, musty	59	0.098	[4,20,27]
32	<i>E,E</i> -2,4-Nonadienal <sup>d</sup>	1705	1221	Green, fatty	57	0.17	
33	Dodecanal <sup>c</sup>	1722	1409	Fatty, citrus	62	0.079	[19]
34	Geraniol <sup>c</sup>	1744	1320	Lemon, minty	63	0.14	[18,20,27]
35	<i>L</i> -Carvone <sup>c</sup>	1751	1254	Spearmint	66	0.010	[4,10,14,19,20]
36	Unknown	1758		Sweet, geranium	54	t	
37	Citronellol <sup>d</sup>	1773	1233	Rose, sour	64	0.073	[4,14]
38	<i>E,E</i> -2,4-Decadienal <sup>c</sup>	1820	1311	Green, fatty	57	0.0067	[4,13,15]
39	$\beta$ -Damascenone <sup>d</sup>	1835	1393	Apples, tobacco	54	0.064	[39]
40	Geraniol <sup>c</sup>	1850	1276	Rose, geranium	54	0.025	[20]
41	Guaiacol <sup>d</sup>	1872	1091	Green, fatty	45	0.028	
42	Unknown	1885		Green, fruity	55	t	
43	$\gamma$ -Octalactone <sup>d</sup>	1936	1262	Fatty, herbal	34	t	
44	$\beta$ -Ionone <sup>d</sup>	1955	1494	Floral, raspberry	74	0.010	[13,15]
45	Unknown	1971		Woody, herbal	48	t	
46	Unknown	2044		Musty, minty	54	t	
47	<i>m</i> -Cresol <sup>d</sup>	2091	1084	Manure, plastic	60	0.0061	
48	Unknown	2169		Wood	44	0.012	
49	Sotolon <sup>d</sup>	2208	1120	Spicy	61	0.018	[28]
50	$\beta$ -Sinensal <sup>c</sup>	2249	1706	Sweet	73	0.018	[18]
51	Wine lactone <sup>d</sup>	2263	1467	Dill, spicy	83	t	[13,15]
52	Unknown	2303		Pepper, wood	64	0.0025	
53	Unknown	2429		Soapy, musty	64	t	
54	Unknown	2542		Soapy, incense	43	t	
55	Nootkatone <sup>c</sup>	2573	1834	Sour fruit,banana	53	0.021	

<sup>a</sup> Averages of normalized intensities evaluated by four panelists in four replications.

<sup>b</sup> Compounds previously reported by GC–O in orange oil [4,18–20]; in orange juice or drink [10,13–15,17,27,28,39].

<sup>c</sup> Identified by linear retention index on DB-wax, DB-5, aroma descriptor as compared with standard, and MS.

<sup>d</sup> As c, but no MS.

<sup>e</sup> Coelutes with limonene on FID.

<sup>f</sup> t, trace, not detected by FID.

recently reported in orange and grapefruit juices [13,15], produced one of the most intense aromas and had not been previously reported in orange essence or peel oil. Other aroma compounds found for the first time in orange essence or peel oil are: *Z*-3-hexenal, 1,8-cineole, 1-octen-3-one, hexanol, *E*-2-octenal, 1-octen-3-ol, *Z*-4-decenal, *E,E*-2,4-nonadienal,  $\beta$ -damascenone, guaiacol,  $\gamma$ -octalactone, *m*-cresol, and sotolon. Several of these were also reported in recent GC–O studies of orange juice [13,15]. However, potent aroma compounds which have not been previously reported in any orange oil or juice are: *E*-2-octenal, 1-octen-3-ol, *Z*-4-decenal, *E,E*-2,4-nonadienal, guaiacol,  $\gamma$ -octalactone, and *m*-cresol.

### 3.2. Identification of orange essence oil volatiles using GC–MS

Ninety-five compounds were detected using GC–MS with a DB-5 column. Seventy-four of these have been identified (Table 2) using the combination of retention index value and mass spectral matching against library standards. Twelve terpenes, 16 sesquiterpenes, 16 alcohols, 15 aldehydes, four ketones, six esters, four terpene and sesquiterpene oxides, and an alkane were identified by GC–MS. Those which could not be confirmed by comparing retention index values and corresponding mass spectra were marked as being tentatively identified.

Earlier compositional studies of orange essence oil employed packed columns. Thus the number of compounds reported in these studies range from 13 [22] to 38 [24]. Therefore, many of the essence oil compounds identified using GC–MS are being reported for the first time. Twenty compounds had not been previously reported in orange essence oil. They are:  $\alpha$ -thujene, camphene,  $\delta$ -3-carene,  $\alpha$ -terpinolene, *Z*-limonene oxide, *E*-limonene oxide,  $\beta$ -terpineol, *p*-cymene-8-ol, piperitone, perilla alcohol, *E,E*-2,4-decadienal,  $\alpha$ -terpinyl acetate, linalyl acetate, *p*-menth-1-en-9-ol acetate, aromadendrene, germacrene D,  $\delta$ -cadinene, germacrene B, caryophyllene oxide, and  $\gamma$ -eudesmol. Most of these had been previously reported in orange or other citrus peel oils. More recent studies on orange and orange hybrid peel oils have reported from 54 [20] to 126 [35] volatiles. Compounds not previously reported in either orange

essence or peel oils are: *Z*-3-hexenal, *E*-2-hexenal, verbenone, *Z*-verbenol, iso-longifolene,  $\gamma$ -caryophyllene, longifolene, *Z*- $\beta$ -guaiane, *E*- $\beta$ -guaiane, and  $\alpha$ -selinene.

Over one third of the components identified by GC–MS are terpenes or sesquiterpenes and they accounted for over 96% of the total FID peak area. This is in accordance with other orange essence oil reports, where they were 88.6% [25], 86.4–98.9% [21], 96.9% [22], and 97.9% [23]. In all cases limonene was by far the major terpene.

Alcohols and aldehydes also accounted for a large number of identified compounds and in particular linalool, octanal, and decanal, produced significant MS total ion current (TIC) peak areas. In previous compositional reports of orange essence oil, linalool was reported as producing between 0.1% [25] and 0.6% [22] of total TIC peak area. Octanal was reported from 0.45% [25] to 1.1% [23] and decanal from 0.28% [23] to 0.7% [22]. In this sample, linalool was found to be at the high end of the range reported in the literature, 0.68%, octanal was found to contribute 0.31% (at the low end of the literature range), and decanal, at 0.32%, was in between reported literature values.

The vast majority of volatiles identified in orange essence oil using GC–MS (49 of 74) (Table 2) had no aroma activity. The 25 compounds identified by GC–MS that did exhibit aroma activity are indicated using bold font. Furthermore, more than 95% of the total volatile material had no aroma activity. Conversely, some volatiles with high aroma activity, for example  $\beta$ -ionone and wine lactone, produced no detectable TIC peak, most likely because of their low concentration. Essentially all the aroma activity in orange essence oil is produced by slightly more than 4% of the total volatiles.

### 3.3. Aroma impact of volatile compounds

Aldehydes are major contributors to orange essence oil aroma. Eleven of the 15 aldehydes detected by GC–MS also had aroma activity. It should also be noted that four potent aldehydes were not detected by GC–MS. Octanal, decanal, citronellal, and  $\beta$ -sinensal produced some of the most intense aromas and were key aroma impact compounds in the oil.

Alcohols also contributed to the oil's aroma.

Table 2

Volatile compounds in orange essence oil determined by GC–MS

No.	Compound <sup>a</sup>	Linear retention index DB-5	Previously reported <sup>b</sup>
1	<b>Z-3-Hexenal</b> <sup>c</sup>	795	
2	<b>Ethyl butyrate</b>	797	[21–23,25]
3	<i>E</i> -2-Hexenal	848	
4	<i>Nonane</i> <sup>d</sup>	896	
5	$\alpha$ -Thujene	923	[35,40,41]
6	<b><math>\alpha</math>-Pinene</b>	934	[4,20–25,35,40,41]
7	Camphene	952	[4,20,35,40]
8	Sabinene	974	[4,20,21,23,25,35,40,41]
9	<b><math>\beta</math>-Pinene</b>	987	[4,20,24,35,40,41]
10	<b>Myrcene</b>	992	[4,20–23,35,40,41]
11	<b>Octanal</b>	1006	[4,20,21,23–25,35,40,41]
12	$\delta$ -3-Carene	1012	[35,40,41]
13	<b>Limonene</b>	1032	[4,20–25,35,40,41]
14	<b><math>\gamma</math>-Terpinene</b>	1071	[4,35,40,41]
15	<b>Octanol</b>	1073	[4,20,21,24,35,40]
16	$\alpha$ -Terpinolene	1096	[4,20,35,40,41]
17	<b><i>p</i>-Cymene</b>	1100	[4,20,21,40,41]
18	<b>Linalool</b>	1106	[4,20–25,35,40,41]
19	<b>Nonanal</b>	1109	[4,20,22,23,35,41]
20	<i>p</i> -Mentha-1,3,8-triene	1123	
21	<i>E</i> - <i>p</i> -Mentha-2,8-dienol	1132	[25,40]
22	<i>Z</i> -Limonene oxide	1144	[4,20,40,41]
23	<i>E</i> -Limonene oxide	1147	[4,20,40,41]
24	<b>Citronellal</b>	1156	[4,20,23,24,35,40,41]
25	$\beta$ -Terpineol	1159	[4,20]
26	Nonanol	1172	[21]
27	1,8-Menthadiene-4-ol	1189	
28	<b>Terpinene-4-ol</b>	1193	[4,24,35,40]
29	<i>p</i> -Cymene-8-ol	1196	[4]
30	$\alpha$ -Terpineol	1207	[21–24,35,40,41]
31	<b>Decanal</b>	1210	[4,20,22–24,35,40,41]
32	Verbenone	1218	
33	Nerol	1221	[4,24,40]
34	<i>E</i> -Carveol	1229	[4,20,21,25,40,41]
35	1- <i>p</i> -Menthene-9-ol	1232	
36	<i>Z</i> -Carveol	1242	[4,25,35,40,41]
37	<i>Z</i> -Verbenol	1244	
38	<b>Geraniol</b>	1252	[4,20,40]
39	<b><i>E</i>-2-Decenal</b>	1266	[4,20,40]
40	<b><i>l</i>-Carvone</b>	1256	[4,20–22,24,25,35,40,41]
41	<b>Neral</b>	1273	[4,20,23,24,35,40,41]
42	Piperitone	1282	[4]
43	Perilla aldehyde	1290	[4,20–22,24,35,40,41]
44	<i>Limonene dioxide</i>	1294	
45	<b>Geranial</b>	1300	[20,22–24,35,40,41]
46	Perilla alcohol	1310	[4]
47	<b><i>E,E</i>-2,4-Decadienal</b>	1323	[4]
48	Citronellyl acetate	1348	[24,40]
49	$\alpha$ -Terpinyl acetate	1354	[4,20,35,40]
50	Linalyl acetate	1359	[4,20,40]
51	Neryl acetate	1376	[20,24,40,41]
52	$\alpha$ -Copaene	1391	[4,21,24,35,40,41]
53	iso-Longifolene	1402	

Table 2. Continued

No.	Compound <sup>a</sup>	Linear retention index DB-5	Previously reported <sup>b</sup>
54	<b>Dodecanal</b>	1411	[4,23,24,35,40,41]
55	<i>γ</i> -Caryophyllene	1414	
56	Longifolene	1416	
57	<i>p</i> -Ment-1-en-9-ol acetate	1424	[20,40]
58	<b>β-Caryophyllene</b>	1439	[4,20,21,23,24,35,40,41]
59	<i>9-epi-Caryophyllene</i>	1453	
60	Aromadendrene	1464	[4]
61	<i>Z</i> -β-Guaiene	1489	
62	<i>Viridiflorene</i>	1494	
63	Germacrene D	1499	[4,20,40,41]
64	<i>E</i> -β-Guaiene	1503	
65	Valencene	1513	[21–24,35,40,41]
66	α-Selinene	1517	
67	δ-Cadinene	1530	[41]
68	7- <i>epi</i> -α-Selinene	1540	[24]
69	Germacrene B	1560	[4]
70	Caryophyllene oxide	1606	[4,40]
71	<i>γ</i> -Eudesmol	1651	[40]
72	<b>β-Sinensal</b>	1700	[23,35,40,41]
73	α-Sinensal	1757	[4,23,24,35,41]
74	<b>Nootkatone</b>	1833	[23,24,35]

<sup>a</sup> Identified by linear retention index on DB-5 and library mass spectra (see Section 2).

<sup>b</sup> Compounds previously reported by GC–MS in orange essence oil [21–25]; other orange oil [4,20,35,40,41].

<sup>c</sup> Compounds in boldface have aroma activity.

<sup>d</sup> Compounds tentatively identified (not very high purity and fit and/or never previously reported) have been italicized.

Sixteen alcohols were detected by GC–MS and 10 alcohols exhibited aroma activity. However, only four of these, octanol, linalool, terpinen-4-ol, and geraniol were detected by both methods. Linalool was one of the key aroma impact compounds.

Although orange essence oil is more than 90% terpenes, few terpenes displayed aroma activity. The aroma activity of these terpenes was generally of low to medium intensity and contributed little to the total aroma. Limonene is the major component and its apparent aroma activity may be due to co-eluting trace components. β-Caryophyllene was the only sesquiterpene with apparent aroma activity, but 15 others were detected by GC–MS. Valencene, which has been used as a quality measure, was present in higher amount than most of the other volatiles (0.83% FID area), but did not exhibit aroma activity. Terpenes and especially sesquiterpenes tend to have high aroma thresholds. Thus, few were detected using GC–O. None of the terpene oxides detected by GC–MS in this study were found to have aroma activity.

The larger esters detected by GC–MS, such as linalyl acetate, citronellyl acetate, neryl acetate, and α-terpinyl acetate, did not have aroma activity. Ethyl butyrate was the only ester detected by GC–MS that also exhibited aroma activity. Two lactones that were not detected by GC–MS exhibited intense aroma activity. One of these, wine lactone, was a key aroma impact compound.

No single compound exhibited the characteristic aroma of orange oil. No aroma active component was described as possessing an orange or orange juice like aroma. The orange essence oil used in this study had an aroma similar to fresh squeezed orange juice. Therefore it is the combination and relative balance of the aroma active compounds which produce orange aroma. Some of the compounds, such as *γ*-terpinene, *p*-cymene, octanal, citronellal, decanal, neral, dodecanal, and geraniol, had citrusy or lemony aromas and they, in combination with the fruity and floral smelling compounds, ethyl butyrate, hexanol, linalool, citronellol, geraniol, β-ionone, and nootkatone, are likely responsible for the top notes of

the orange oil aroma. The minty and green smelling compounds, respectively *l*-carvone and most of the terpenes, and the unsaturated aldehydes are likely to be bottom notes and balancing components. A few compounds had spicy, herbal, or musty smells and contribute to the balancing, with wine lactone and sotolon having the most intense aromas.

Some of the key aroma impact compounds were present in appreciable amounts, notably linalool, 0.68% peak area, octanal, 0.31%, and decanal, 0.32%. However, citronellal, at 0.04% and  $\beta$ -sinensal, 0.02% were in low concentration and  $\beta$ -ionone (0.01%) and wine lactone (trace) were not detectable by typical GC–MS.

Trace components can have major aroma activity in food products. Wine lactone has one of the lowest aroma thresholds ever reported, 0.04 parts per trillion in air [36], and need not be present in quantities detectable by traditional GC–MS or GC–FID to have large aroma impact. Octanal, decanal, and linalool have low thresholds of 0.1–6 ppb in water [37] and are present in relatively high amounts in orange essence oil. Citronellal and  $\beta$ -sinensal have low ppb range thresholds as well and  $\beta$ -ionone has the very low threshold of 0.007 ppb [37]. In fact most of the aroma active compounds detected have low aroma thresholds, generally ranging from 0.1 to 50 ppb. *E,E*-2,4-nonadienal and *E,E*-2,4-decadienal have thresholds lower than 0.1 ppb and sotolon,  $\beta$ -damascenone, and 1-octen-3-one have thresholds lower than 0.01 ppb [37].

#### 4. Conclusions

GC–MS can be used to identify those compounds present in highest concentrations, but it is clear from these results that GC–O is an important tool to determine the aroma active compounds in food or flavor samples. In this study, more compounds were detected using GC–MS (95) than by GC–O (55). Most of the compounds identified using GC–MS were not aroma active; only 4% of the total volatile material in orange essence oil has aroma activity. Quality models based on statistical correlation of quantitative compositional data have been largely unsuccessful [38] because as shown in this study, most do not contribute to the products' aroma. The

aroma active compounds directly identified in this study are more likely to have success because each component in the model will directly influence flavor and not simply correlate with it.

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